

Therapeutic Drug Monitoring of Posaconazole in Allogeneic Hematopoietic Stem Cell Transplantation Patients Who Develop Gastrointestinal Graft-versus-Host Disease

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Posaconazole (PCZ) is the latest triazole antifungal agent that has been approved for prophylaxis of invasive aspergillosis in high-risk immunocompromised patients, such as allogeneic hematopoietic stem cell transplantation patients, who develop graft-versus-host disease (GVHD). PCZ has high interindividual variability with regard to its plasma drug trough concentrations (C_{\min}). Moreover, the concentration–efficiency relationship remains to be better characterized in prophylaxis. To determine the variability factors in plasma drug concentrations, the PCZ C_{\min} and clinical parameters (localization of GVHD, presence of diarrhea, and diagnosis of invasive aspergillosis) were collected retrospectively in 29 consecutive allogeneic hematopoietic stem cell transplantation patients who developed GVHD and were receiving prophylactic PCZ (200 mg, 3 times/day, for ≥ 7 days). Blood samples were analyzed at steady state to determine the PCZ C_{\min} by liquid chromatography–tandem mass spectrometry. The average PCZ C_{\min} was 1.28 ± 0.82 mg/liter (mean \pm standard deviation; $n = 292$ dosages), with an intraindividual variability of 49% and an interindividual variability of 64%. Twenty percent of C_{\min} s were below 0.7 mg/liter, which is considered the threshold of efficacy by the Food and Drug Administration. The patients who had gastrointestinal (GI) GVHD experienced a 24% reduction in the posaconazole C_{\min} , compared with those with other localizations of GVHD. This decrease reached 33% when patients presented with diarrhea due to GI GVHD or an infectious etiology. PCZ C_{\min} s were 26% lower when invasive aspergillosis was declared. These data demonstrate that GI disturbances affect drug concentrations. Thus, therapeutic monitoring of PCZ can be used to detect low drug concentrations, possibly resulting in a lack of efficacy of invasive aspergillosis prophylaxis.

Despite the availability of new antifungal agents, invasive aspergillosis (IA) remains a leading cause of morbidity in immunocompromised patients. Posaconazole (PCZ) is the most recent broad-spectrum triazole antifungal agent to be approved for use to prevent IA in high-risk immunocompromised patients. In graft-versus-host disease (GVHD), a frequent complication after allogeneic stem cell transplantation, patients who receive PCZ prophylaxis have a lower cumulative incidence of proven or probable IA than those who are given fluconazole (19).

Until now, the only way of administering PCZ was a 40-mg/ml oral suspension, which led to wide variations in bioavailability (17). Based on its lipophilicity, high-fat-content meals improve its absorption rate, leading to the recommendation of taking PCZ with at least a regular meal (4, 12). Because the gastric pH value also influences drug absorption, the concomitant intake of a PCZ oral suspension with an acidic beverage increases its absorption (12, 14).

Furthermore, due to its saturable absorption, bioavailability rises when the daily dose is split, i.e., for prophylactic treatment with 200 mg three times a day (t.i.d.) (6). Because the bioavailability of a PCZ oral suspension depends highly on food intake, the frequency of administration, and gastric pH, trough plasma PCZ concentrations vary widely in healthy volunteers (4, 12) and different patient populations, including neutropenic stem cell transplant recipients (11, 18).

PCZ exposure affects treatment outcomes in refractory IA (20) and as prophylaxis in allogeneic hematopoietic stem cell transplantation (HSCT) patients (19). A relationship between PCZ exposure and efficacy was proposed in a study of a curative strategy

(20), but it was associated with marked kinetic variability. Little data are available with regard to PCZ as a prophylactic agent.

Allogeneic HSCT recipients who develop GVHD and receive prophylactic PCZ are susceptible to gastrointestinal (GI) GVHD and other gut complications, such as infectious and toxic diarrhea, which create variations in trough drug concentrations (13). Few studies have performed longitudinal therapeutic drug monitoring (TDM) of PCZ plasma concentrations (2, 9, 13, 18). Only longitudinal PCZ TDM provides information that can be used to identify factors that influence plasma trough drug concentrations and thus improve therapeutic management of patients. Moreover, there are limited data on PCZ TDM beyond clinical licensing trials, and we speculate that the variability in PCZ concentrations differs more than would be found in such rigorous protocol-driven settings.

Thus, the purpose of this study was to determine the impact of clinical status on the variability of PCZ plasma trough concentrations in an unselected prospective group of allogeneic HSCT recipients who developed GVHD and received prophylactic PCZ.

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MATERIALS AND METHODS

Patients. This retrospective study was conducted at the Grenoble University Hospital. The patient population was limited to recipients of HSCT who developed GVHD. Any adult patient who was admitted between January 2010 and January 2011, presented with GVHD post-HSCT, received PCZ for IA prophylaxis (200 mg, 3 times/day, ≥ 7 days), and had available plasma PCZ trough concentrations (C_{\min}) was included in the study. Patients were classified according to the location of their GVHD, and no distinction was made with regard to acute or chronic GVHD. All patients received corticotherapy for GVHD (prednisone at 2 mg/kg/day for acute GVHD and 1 mg/kg/day for chronic GVHD). To improve the absorption of PCZ, patients were asked to take their PCZ with an acidic beverage. All patients gave written consent for collection and use of their data.

Study design and clinical data collection. The posaconazole C_{\min} was monitored at each weekly outpatient visit. Patients were asked not to take their treatment on the morning they came for their day care hospitalization, and they directly underwent blood sampling before administration of PCZ. This study was an observational evaluation, and the primary outcome was the impact of clinical status on the PCZ C_{\min} . Interindividual variability for the PCZ C_{\min} was determined in the 29 patients in this study. The percentage of patients who achieved the 0.7-mg/liter target concentration, which is considered the efficacy threshold by the FDA (8), was also calculated.

The patients were first stratified by the organs that were involved in their GVHD: GI ($n = 14$) or non-GI ($n = 15$). Clinical status information, i.e., symptoms of GVHD, diarrhea, mucositis, and diagnosis of IA, was collected retrospectively for each PCZ C_{\min} data point by using patient medical records, which were completed by clinicians when patients came for their weekly outpatient visit. The potential impact of diarrhea or the presence of IA on the PCZ C_{\min} was then assessed, creating 4 subgroups *a posteriori* according to the clinical status of the patient when the PCZ C_{\min} was determined, based on the presence or absence of diarrhea and declared or undeclared IA.

Blood samples. Patients came weekly for their outpatient visit and submitted blood samples for PCZ TDM. Blood samples were collected at steady state to measure the PCZ C_{\min} . With respect to the elimination half-life of PCZ (20 h), the first sample was taken at least 7 days after initiation of the treatment.

PCZ plasma concentration. PCZ concentrations were measured by a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method with an online sample cleanup assay. After protein precipitation (100 μ l of plasma plus 100 μ l ultrapure water and 200 μ l methanol-acetonitrile [50:50, vol/vol], and 0.1% perchloric acid containing the internal standard, deuterated D3-PCZ at 1,000 ng/ml), 10 μ l of the supernatant was injected into a two-dimensional chromatographic system (Prominence LC-20; Shimadzu Kyoto, Japan).

The first step comprised an online sample cleanup with an aqueous solution, which containing 3.0% methanol and 0.1% formic acid, at 4 ml/min on a perfusion chromatography column (POROS R1/20; 20 μ m; 2.1 mm by 30 mm; Applied Biosystems, Foster City, CA). The extract was then eluted at 0.5 ml/min in backflush mode and transferred to the analytical column (Phenomenex Luna; 5 μ m; phenyl-hexyl; 2 mm by 50 mm; Torrance, CA), maintained at 60°C. The elution gradient consisted of 2 mobile phases (phase A, 10 mM ammonium formate at pH 3 after addition of formic acid; phase B, acetonitrile-methanol [70/30, vol/vol] and 0.1% acetic acid).

Compounds were detected by tandem mass spectrometry (API 3200QTRAP; ABSciex, Foster City, CA) with positive electrospray ionization and the following multiple reaction monitoring ion transitions: m/z 701.3/127.1 for PCZ and m/z 704.3/130.1 for the deuterated internal standard (D3-PCZ). The plasma drug standard curve ranged from 0.1 to 20 mg/liter. The between-day coefficients of variation were 6.05% and 4.66% for target concentrations of 0.8 mg/liter and 3 mg/liter, respectively, and

TABLE 1 Underlying hematological diagnoses and conditioning regimens

Diagnosis or regimen	No. of patients (%)
Hematological malignancies	
Acute myeloid leukemia	10 (34)
Acute lymphoblastic leukemia	6 (21)
Multiple myeloma	5 (17)
Non-Hodgkin lymphoma	5 (17)
Myeloproliferative syndrome	1 (3)
Chronic lymphocytic leukemia	1 (3)
Hodgkin lymphoma	1 (3)
Conditioning regimens	
Myeloablative	8 (28)
Nonmyeloablative	21 (72)
Immunosuppressive therapies	
Cyclosporine + methotrexate	8 (28)
Cyclosporine + mycophenolate mofetyl	21 (72)

the within-day coefficients of variation were 1.01% and 1.09% for target concentrations of 0.8 mg/liter and 1.09 mg/liter, respectively.

Breakthrough assessment of invasive aspergillosis. IA was considered proven, probable, or possible, according to the 2008 European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group criteria (EORTC/MSG) (5). Antigenemia was determined using the *Aspergillus* galactomannan test.

Statistical analysis. Continuous data were expressed as mean \pm standard deviations (SD), and categorical data were expressed as percentages. Bivariate analyses were performed using Fischer's exact test, and the chi-square test was used to compare categorical variables. Comparisons between groups by location of GVHD were analyzed by using the Student *t* test for normally distributed continuous variables (age and body mass index) and the unpaired Mann-Whitney test for the PCZ C_{\min} .

The potential impact of GI GVHD, diarrhea, and age on the PCZ C_{\min} was assessed by regression analysis. First, simple regression between the PCZ C_{\min} and each clinical condition was performed. Then, multiple regression was performed between the PCZ C_{\min} and statistically significant clinical factors in a simple regression analysis.

Comparisons of the PCZ C_{\min} according to diarrhea clinical status in both GVHD groups in the absence of declared IA were analyzed by using two-way analysis of variance (ANOVA); factor A was the location of GVHD (GI or not), and factor B was the presence or absence of diarrhea (D+ or D-). Finally, comparisons of the PCZ C_{\min} by clinical status of diarrhea and IA were made by two-way ANOVA; factor A was the presence or absence of diarrhea (D+ or D-), and factor B was declared (IA+) or absent (IA-) IA. Bonferroni *post hoc* analysis was performed for significant ANOVA results. A *P* value of <0.05 was considered statistically significant. Statistical analysis was performed using Sigma-Stat, version 3.11 (Systat Software, Illinois).

RESULTS

Patient characteristics. Table 1 reports the underlying hematological diagnosis, conditioning regimens, and immunosuppressive therapies of the 29 allogeneic HSCT patients who developed GVHD. All patients received concomitant pump proton inhibitors for prophylaxis of stress ulcers.

Patient characteristics are presented in Table 2 for the entire population and after stratification by location of GVHD: GI (52%) or other (48%), primarily cutaneous. Patients with GI GVHD ($n = 15$) were classified as follows: grade 2 ($n = 10$), grade 3 ($n = 1$), and grade 4 ($n = 4$), according to the consensus con-

TABLE 2 Demographics, baseline clinical characteristics, and PCZ C_{\min} s for four patients who developed invasive aspergillosis while receiving PCZ treatment

Age (yrs)	Sex	Baseline body wt (kg)	No. of C_{\min} dosages performed per patient	PCZ C_{\min} ^a (% CV)	D/IA status (no. of C_{\min} dosages performed) ^b	PCZ C_{\min} (% CV) after D or IA status change
63	F	57	32	0.97 ± 0.41 (43)	D ⁻ /IA ⁻ (23) D ⁻ /IA ⁺ (2) D ⁺ /IA ⁻ (7)	1.12 ± 0.39 (35) 0.70 ± 0.14 0.57 ± 0.22 (39)
60	F	42	15	1.03 ± 0.44 (43)	D ⁻ /IA ⁻ (5) D ⁻ /IA ⁺ (2) D ⁺ /IA ⁻ (6) D ⁺ /IA ⁺ (2)	1.52 ± 0.31 (20) 1.05 ± 0.07 0.63 ± 0.21 (33) 0.95 ± 0.07
54	M	100	13	1.26 ± 0.73 (58)	D ⁻ /IA ⁻ (10) D ⁻ /IA ⁺ (2) D ⁺ /IA ⁻ (1)	1.55 ± 0.55 (36) 0.20 ± 0.14 0.50
56	M	78	1	0.20	D ⁺ /IA ⁺ (1)	0.20

^a C_{\min} values are in mg/liter, and means ± SD are presented. CV, coefficient of variation (reported as a percentage).

^b D, diarrhea. The number of C_{\min} dosages performed before change in D/IA status is shown. When there were only 1 or 2 data points (C_{\min} dosages performed for D/IA status), a CV was not calculated.

cerning acute GVHD (16). Of those with GI GVHD, 8 became chronic according to the consensus on chronic GVHD (7), and 4 of them developed extensive disease. At baseline, 1 patient from the non-GI GVHD group presented with chronic mucositis. During follow-up, 4 patients declared a possible or probable IA infection according to the EORTC criteria, 3 of whom were in the GI GVHD group.

Posaconazole trough concentrations. A total of 292 PCZ C_{\min} s were measured during routine TDM of the 29 patients (range per patient, 1 to 33; median, 8). Figure 1 shows the mean concentration for each patient ($n = 29$), regardless of clinical status. The overall mean PCZ C_{\min} was 1.28 ± 0.82 mg/liter ($n = 292$), and the median value was 1.10 mg/liter (range, 0.10 to 4.70). PCZ C_{\min} had an interindividual variability of 64%. Considering all concentrations, 12/292 (4%) were beyond the lower limit of quantification of the analytical method (0.1 mg/liter), and 58/292 (20%) were lower than 0.7 mg/liter. Of the 29 patients, 3 (10%) had a mean C_{\min} of <0.7 mg/liter (Fig. 1). The mean C_{\min} for patients was 1.20 ± 0.65 mg/liter, and the median C_{\min} was 1.07

mg/liter (range, 0.50 to 2.08). The intraindividual variability in the PCZ C_{\min} was $49\% \pm 18\%$.

Posaconazole trough concentrations according to clinical status. By simple regression analysis, we detected a significant relationship between the PCZ C_{\min} and age ($r = 0.309$; $P < 0.0198$), the presence of GI GVHD ($r = 0.22$; $P < 0.001$), and the occurrence of diarrhea ($r = 0.433$; $P < 0.001$). Despite the low regression coefficients, multiple regression analyses indicated that age ($P = 0.014$) and diarrhea ($P < 0.001$) affected the PCZ C_{\min} .

Invasive aspergillosis. Four patients (17%) developed probable (3/4) or possible (1/4) IA during prophylaxis with PCZ. Their demographic and baseline clinical characteristics and PCZ C_{\min} s are reported in Table 2. Most patients who developed IA presented with GI GVHD (Table 3). At the time of IA, the corresponding PCZ C_{\min} was 26% lower than that of unaffected patients (C_{\min} , 0.99 ± 0.49 and 1.32 ± 0.85 mg/liter, respectively, when IA was declared or not; $P < 0.05$).

TABLE 3 Patient characteristics, clinical status, and PCZ C_{\min} value by location of GVHD

Characteristic	All patients	Non-GI GVHD	GI GVHD	<i>P</i> value ^a
<i>n</i> (% of total)	29	14 (48)	15 (52)	
Male/female	18/11	10/4	8/7	
Age (yrs; mean ± SD)	48.6 ± 10.8	45.6 ± 8.6	51.3 ± 12.1	<0.05
Weight (kg; mean ± SD)	65.9 ± 14.7	66.5 ± 8.5	64.1 ± 2.4	NS
BMI (kg/m ²) (mean ± SD)	21.7 ± 0.6	21.5 ± 3.5	21.9 ± 2.5	NS
No. (%) with chronic mucositis	1 (3)	1 (7)	0	
No. (%) declaring IA	4 (14)	1 (7)	3 (20)	0.36
PCZ C_{\min} (mg/liter; mean ± SD)	1.28 ± 0.82	1.42 ± 0.86	1.08 ± 0.72	<0.001
PCZ C_{\min} for patient group				
Total	292	169	123	
With diarrhea	88 (30)	7 (4)	81 (66)	<0.001
With IA	36 (12)	7 (4)	29 (24)	<0.001
With diarrhea and IA	7 (2)	0	7 (6)	0.006
No. (%) of patients with PCZ C_{\min} of <0.7 mg/liter ^b				
Total	58 (20)	24 (14)	34 (28)	0.007
With diarrhea	33 (57)	4 (16)	29 (85)	0.001
With IA	8 (14)	2 (8)	6 (18)	0.45
With diarrhea and IA	3 (5)	0 (0)	3 (8)	0.26

^a *P* values correspond to the comparison between the GI GVHD and other location GVHD groups. NS, not significant.

^b FDA threshold of efficacy.

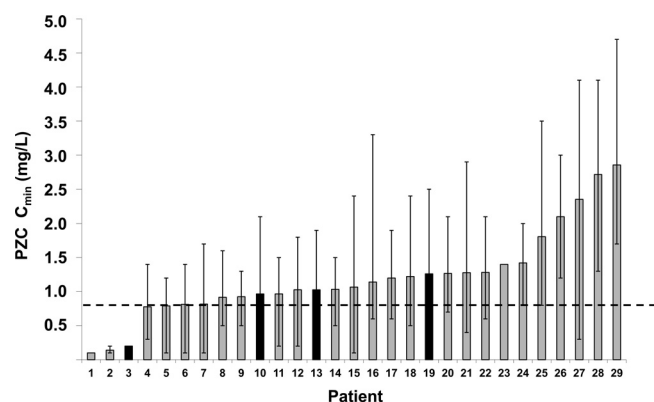


FIG 1 Mean PCZ C_{\min} value for each patient ($n = 29$), independent of clinical status. The histogram bars represent individual means, and histogram lower and upper limits represent minimal and maximal C_{\min} s, respectively. Black histogram bars represent patients with breakthrough infection (invasive aspergillosis). Dashed line, FDA-recommended efficacy threshold (C_{\min} , 0.7 mg/liter).

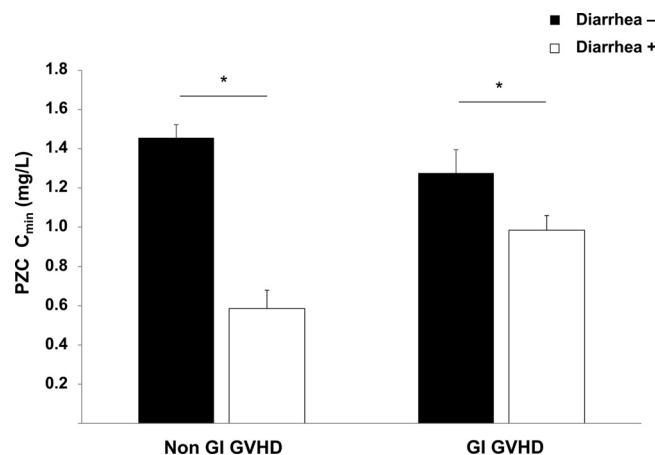


FIG 2 PCZ C_{\min} by clinical status of diarrhea in patients who did not declare invasive aspergillosis, stratified by location of GVHD. *, $P < 0.05$.

GI GVHD and diarrhea. Patients were divided into 2 groups by location of their GVHD. Half of the patients ($n = 15$ patients, $n' = 123$ PCZ C_{\min} data points) presented with GI GVHD. In patients with other locations of GVHD, 7 of 169 PCZ C_{\min} s (4%) were measured when diarrhea was present due to infectious disease (e.g., *Giardia intestinalis* or cytomegalovirus). Among the 123 PCZ C_{\min} data points measured when GI GVHD was declared, 6 (5%) were concomitant with a CMV infection. In the other cases, no infection was reported, and the diarrhea was attributed to GI GVHD. Despite their older age, patients in the GI GVHD group did not differ in weight or body mass index. GI GVHD was associated with a 24% lower PCZ C_{\min} compared with non-GI GVHD levels (Table 3 and Fig. 2). In addition, the percentage of C_{\min} s values that were <0.7 when the GVHD was GI related was 2-fold higher than for other locations. The proportions of PCZ C_{\min} s that were associated with the presence of diarrhea or declared IA were significantly higher in patients who presented with GI GVHD (Table 3). In patients with GI GVHD, half of the measured PCZ C_{\min} s (49%) were below the FDA-recommended value of 0.7 mg/liter, whereas with other locations of GVHD, more than 80% of values exceeded this threshold.

Diarrhea occurred primarily in the GI GVHD group, for which 92% of PCZ C_{\min} s when diarrhea was present were measured (Table 3). When affected by diarrhea, patients experienced a 33% decrease in the PCZ C_{\min} (1.42 ± 0.84 to 0.95 ± 0.66 mg/liter; $P < 0.001$ [$n = 29$ patients]). Figure 2 shows the effects of diarrhea on the PCZ C_{\min} in GI and non-GI GVHD patients (two-way ANOVA, with the factor diarrhea, $P < 0.05$; with the factor GVHD location, $P = 0.52$, and for the interaction, $P = 0.09$). The effect of diarrhea was also observed on the PCZ C_{\min} regardless of IA status (Fig. 3) (two-way ANOVA for the factor diarrhea, $P < 0.05$; with the factor IA, $P = 0.06$; interaction, $P = 0.47$). In the absence of diarrhea, 18% of PCZ C_{\min} s were under the 0.7 mg/liter threshold, compared with 49% among those with diarrhea.

Figure 4 illustrates the influence of diarrhea on intraindividual variations in drug concentration. Patient A had a mean PCZ C_{\min} of 1.01 ± 0.35 mg/liter with a coefficient of variation of 34%. The mean drug concentration in the presence of diarrhea was 41% lower than when diarrhea was not reported (0.70 ± 0.35 mg/liter and 1.19 ± 0.30 mg/liter, respectively). This patient did not de-

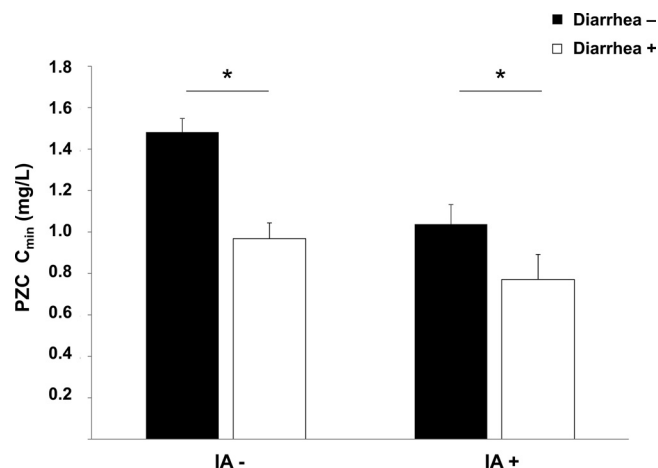


FIG 3 PCZ C_{\min} by clinical status of diarrhea and invasive aspergillosis. *, $P < 0.05$.

clare IA. Patient B had a mean PCZ C_{\min} of 1.03 ± 0.44 mg/liter, with a coefficient of variation of 43% (the second patient in Table 2). This patient developed IA which, with GVHD, caused the patient's death. For this patient, diarrhea induced a 59% decline in the PCZ C_{\min} (1.52 ± 0.31 to 0.63 ± 0.21 mg/liter). The declaration of IA followed low PCZ C_{\min} results. Thus, the decrease in PCZ concentration could have caused the declaration of IA.

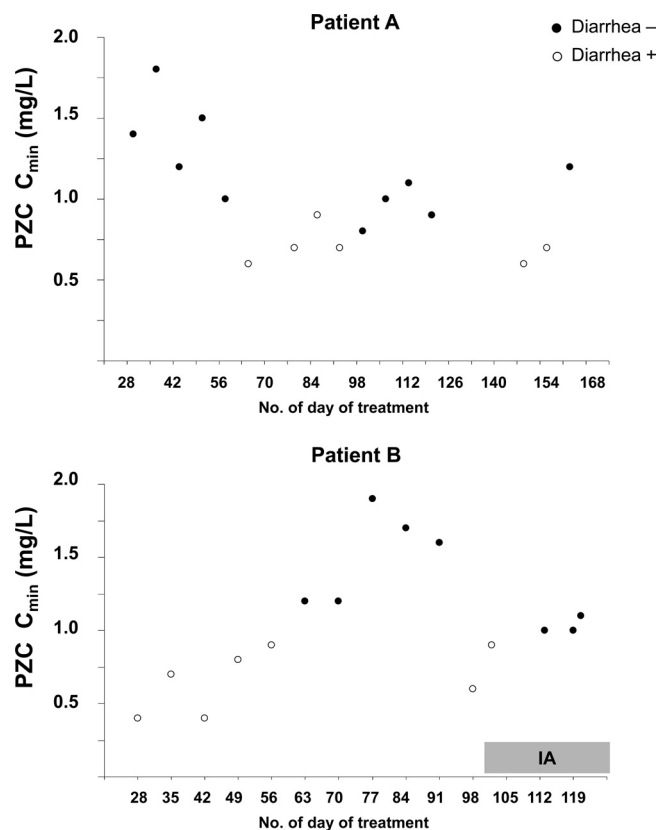


FIG 4 Individual illustrative evolution of the PCZ C_{\min} , depending on clinical status of diarrhea and invasive aspergillosis in 2 patients: one without IA (patient A) and the second who declared IA (patient B).

DISCUSSION

This study reports longitudinal TDM of PCZ for antifungal prophylaxis in HSCT recipients who have developed GVHD. We observed high intra- and interindividual variability in PCZ C_{min} in this group of patients, and the patent influence of clinical status, i.e., GI GVHD, diarrhea, and IA on the PCZ C_{min} . Optimizing the absorption and maintaining adequate concentrations of PCZ are essential for preventing IA in HSCT patients.

The recommended target of 0.7 mg/liter, proposed by the FDA (8), correlates with clinical outcome (11, 13). In our study, 20% of the 292 measured concentrations were <0.7 mg/liter, contrasting with reported values of ~44% for <0.5 mg/liter (13); 70.3% (17) and 90.5% (2) levels were <0.7 mg/liter. Moreover, our mean (1.28 ± 0.82 mg/liter) and median (1.10 mg/liter) PCZ C_{min} s and the initial steady-state levels (1.42 ± 0.82 mg/liter) were higher than those of earlier studies (2, 3, 11, 13, 17).

Strategies that optimize PCZ absorption include its administration with or after a high-fat meal, with enteral nutritional supplements, with an acidic beverage, in divided doses, and without acid-suppressing drugs (12). In our study, patients were instructed to take PCZ with an acidic beverage and to divide their daily dose to take it 3 times per day. These recommendations might explain the higher PCZ C_{min} that we observed compared with others, who did not report specific conditions of administration.

Intake of a prophylactic treatment for a stress ulcer reduced the PCZ C_{min} , as fewer patients reached the minimal target of 0.5 mg/liter used by Bryant et al. (2) and proposed initially by Andes et al. (1). Although all of our patients received such prophylaxis, the concomitant intake of PCZ with an acidic beverage effected high plasma concentrations of PCZ, suggesting the importance of taking PCZ with an acidic beverage to maximize its absorption and exposure.

Despite the desire to optimize absorption, wide inter- (64%) and intraindividual (49%) variabilities in PCZ C_{min} values remained, as reported previously (18). This can be explained by other factors, such as age, which has previously been demonstrated (9, 15). Considering the clinical status of GI GVHD and diarrhea, for each measure of PCZ C_{min} , a significant relationship was noted between the PCZ C_{min} and each of these factors.

In patients who had GI GVHD, the PCZ C_{min} was lower than in patients with other locations of GVHD. The influence of acute GVHD on the PCZ C_{min} was demonstrated in a large study of HSCT recipients with GVHD (19). Nevertheless, Lebeaux et al. failed to detect a significant relationship with GVHD, but they did with diarrhea (13). In our study, diarrhea induced a 33% decrease in the PCZ C_{min} , regardless of location of GVHD, but occurring primarily in the GI GVHD group, consistent with data on other HSCT patients in whom diarrhea caused the C_{min} to fall below 0.5 mg/liter more frequently (13) and for PCZ concentrations to decrease by 40% (10, 11, 19).

The occurrence of diarrhea reduced the C_{max} from 1.423 to 0.623 mg/liter and the C_{min} from 0.989 to 0.609 mg/liter (11). These closed values of maximum and residual concentrations when diarrhea was present are in favor of reduced absorption when patients present with diarrhea. This finding could be explained by a reduced time of contact of the molecule with the digestive wall, limiting its absorption. Using a population pharmacokinetics model, Kohl et al. demonstrated that diarrhea in-

duced a 59% loss in bioavailability of PCZ in HSCT patients (9). In a recent study of patients from general practice, no effect of diarrhea was reported on the PCZ C_{min} , which could be explained by the low PCZ C_{min} s reported, even in the absence of diarrhea (2).

Nevertheless, in the group of patients who reached a concentration of 0.5 mg/liter, none with diarrhea reached this target, whereas in the absence of diarrhea, 5 of 16 managed to attain this target (2). Notably, diarrhea occurred almost exclusively in patients with GI GVHD. Overall, data from our longitudinal TDM program, with findings from other clinical trials, strongly support that in patients who present with gastrointestinal disturbances, the PCZ C_{min} decreases and should be monitored carefully.

Although the relationship between therapeutic response and the PCZ C_{min} has been demonstrated when PCZ is used as a curative treatment, it is unknown whether it exists when PCZ is used as a prophylactic agent. Walsh et al. demonstrated that more than 75% of patients who were treated for IA with PCZ developed a global response to the treatment (20) when the PCZ C_{min} was >1.25 mg/liter. Our data are in favor of a relationship between C_{min} level and prophylactic efficacy. In patients who declared an IA, the PCZ C_{min} was lower than that in the absence of IA. In other studies that evaluated prophylactic PCZ, median PCZ C_{min} was lower in patients with IA, albeit not significantly (12, 13). Further, in cases with declared IA, the low PCZ C_{min} did not decline further on the occurrence of diarrhea. A low C_{min} could thus be a cause of the development of IA, as illustrated in patient B. Nevertheless, the association between PCZ C_{min} and prophylactic efficacy requires further examination.

We acknowledge several limitations of our study, including its retrospective design and the small number of patients. Moreover, the low occurrence of chronic mucositis GVHD did not allow us to compare the PCZ C_{min} in the presence or absence of mucositis, although this factor appears to cause a decrease in the PCZ C_{min} (13). Because patients were not hospitalized continuously, the monitoring of concomitant intake of PCZ with an acidic beverage was not performed. Nevertheless, all patients received specific education by clinicians and a clinical pharmacist during their hospital stay for HSCT. During each weekly day care visit of their follow-up, a clinician asked patients specifically regarding their compliance of concomitant administration of PCZ with an acidic beverage.

Our study, performed in HSCT recipients who developed GVHD from general practice, demonstrates that a high PCZ C_{min} can be achieved when PCZ is administered with an acidic beverage. Gastrointestinal disturbances, such as GI GVHD and diarrhea, clearly affect drug concentrations, possibly decreasing the efficacy of IA prophylaxis. These data suggest that PCZ plasma drug concentrations should be monitored in HSCT recipients who develop GVHD to prevent the occurrence of IA, possibly consecutive to low drug concentrations, in cases of gastrointestinal disturbances. Subsequent studies focusing on TDM in patients who receive PCZ for invasive fungal infection prophylaxis should include an evaluation of the therapeutic strategy in cases with low PCZ C_{min} s, particularly when IA is suspected.

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